BACILLUS THURINGIENSIS CHROMOSOMAL GENOME SEQUENCES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C §119(e) of U.S. Provisional Application Ser. No. 60/154,678 filed on Sep. 17, 1999, the entire content of which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to nucleic acid sequences from *Bacillus thuringiensis* and, in particular, to chromosomal genomic DNA sequences. The invention encompasses nucleic acid molecules present in non-coding regions as well as nucleic acid molecules that encode proteins and fragments of proteins. Nucleic acid sequences that encode proteins and/or enzymes and homologues and fragments thereof are encompassed by the invention including but not limited to insect inhibitory proteins, proteins capable of conferring antibiotic resistance, microbial inhibitory proteins including bactericidal, bacteriostatic, fungicidal, and fungistatic proteins, polyketide synthases, transposons and mobile genetic elements and their corresponding transposases, excisases and integrases, phage and phage particle proteins, other useful protein homologues, ribosomal RNA (rRNA), and transfer RNA (tRNA). In addition, proteins and fragments thereof so encoded and antibodies capable of binding the proteins are encompassed by the present invention. The invention also relates to methods of using the disclosed nucleic acid molecules, proteins, fragments of proteins, and antibodies, for example, for gene identification and analysis, preparation of constructs, transformation of cells with nucleotide compositions disclosed herein to produce *Bacillus thuringiensis* proteins or fragments thereof, in particular novel insect inhibitory, bactericidal, fungicidal and nematicidal proteins.

BACKGROUND OF THE INVENTION

[0003] *Bacillus thuringiensis* is a spore-forming Gram-positive bacterium. During sporulation, *B. thuringiensis* produces proteinaceous inclusions which are composed of proteins known as insecticidal crystal proteins (ICPs), Cry proteins, or delta-endotoxins. These proteins are toxic to a variety of insect species including orders Lepidoptera, Coleoptera, Diptera, Hemoptera, Hymenoptera, Orthoptera, and Mallophaga and to nematodes, mites, and protozoa (Beegle and Yamamoto, *Can. Entomol.* 124:587-616; Feitelson, Advanced Engineered Pesticides (L. Kim, ed.), Marcel Dekker, Inc., New York (1993), pp. 63-71; Feitelson, et al., *Bio/Technology* 10:271-275; U.S. Pat. No. 4,948,734 (1990)). Due to their high specificity for particular insect pests and their safety for man and the environment, ICPs have been used as biopesticides for the last three decades. Using molecular genetic techniques, numerous delta-endotoxin genes have been isolated and their DNA sequences determined. The cloning and sequencing of a number of δ-endotoxin genes from a variety of *B. thuringiensis* strains has been described and are summarized by Schnepf et al. (*Microbiol. Mol. Biol. Rev.* 62:775-806, *Bacillus thuringiensis* And Its Pesticidal Crystal Proteins, 1998). The nomenclature and appearance of newly identified genes is summarized and regularly updated at http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/. These genes have been used to develop certain genetically engineered *B. thuringiensis* products that are in commercial use. Recent developments have seen new δ-endotoxin delivery systems developed, including genetically engineered plants that contain and express δ-endotoxin genes. *Bacillus thuringiensis* is a key source of genes, which when modified can be used for transgenic expression to provide pest resistance in plants.

[0004] *B. thuringiensis* strains are classified into subspecies or varieties, based on biochemical and serological criteria (de Barjac, *Entomophaga* 7: 5-61 (1962); de Barjac, *Proceedings of the IIIrd International Colloquium on Invertebrate Pathology* (C. C. Payne and H. D. Burges, eds.), Society for Insect Pathology, U.K., 451-453 (1982)). Each subspecies may produce one or several insecticidal protein toxins. To date, approximately 172 δ-endotoxins belonging to 28 classes have been identified. There is also a nonprotein toxin, the β-exotoxin, secreted by some *B. thuringiensis* strains. This toxin, which is assayed on house fly larvae (Sêbesta et al., "*Thuringiensin*, the δ-exotoxin of *Bacillus thuringiensis*," in W.H. Burgess (ed.), *Microbial Control of Pests and Plant Diseases,* 1970-1980, Academic Press, Inc., New York, pp. 249-281 (1981)), is not as selective as the δ-endotoxins.

[0005] Extensive studies have been carried out with *B. thuringiensis* subspecies that produce proteinaceous inclusions during sporulation. The inclusions are often bipyramidal, but some are cuboidal or multifaceted, and there is a wide variety of other morphologies. Some strains contain more than one type of inclusion in each cell. These inclusions are present within the mother cell adjacent to the spore, but in a few subspecies, they are localized within the exosporium (Aronson et al., *Bacteriol. Rev.* 40:360-402 (1976)). Inclusions are released, as is the spore, upon cell lysis.

[0006] *Bacillus* strains can have a chromosomal genome size of 2.4 to 5.7 Mbp (Carlson, et al., Appl. Environ. *Microbiol.* 60: 1719-1725 (1994)). Physical maps of chromosomes of two *B. thuringiensis* strains, *B. thuringiensis* subsp. Berliner 1715 and *B. thuringiensis* subsp. *B. thuringiensis* HD2, have been constructed and are estimated to be between 5.4 and 5.7 Mbp (Carlson, et al., *Microbiol.* 142: 1625-1634 (1996); Carlson and Kolstø, J. Bacteriol. 175: 1053-1060 (1993)). The total genomes of each of these two strains consist of one or more chromosomes, and a more variable component comprised of extrachromosomal elements (Carlson and Kolstø, *Mol. Microbiol.* 13:161-169 (1994)).

[0007] Most *B. thuringiensis* isolates have several extrachromosomal elements, some of them circular plasmids and others linear (Carlson, et al., *Microbiol.* 60: 1719-1725 (1994)). In general, crystal-protein genes are localized on large plasmids (ca. 40 to 200 Mda) of *B. thuringiensis* (Gonzalez, et al., *Plasmid* 5: 351-365 (1981); Carlton and Gonzalez, Molecular Biology of Microbial Differentiation, American Society for Microbiology, Washington, D.C. 246-252 (1985), Kronstad, et al., J. Bacteriol. 154: 419-428 (1983)), and in some cases, more than one gene is present on a given plasmid (Aronson et al., *Bacteriol. Rev.* 40:360-402 (1976); Carlton et al., "The genetics and molecular biology of *Bacillus thuringiensis*," in D. A. Dubnau (ed.), *The Molecular Biology of the Bacilli*, Vol. II, Academic Press, Inc., New York, pp. 211-249 (1985)). However, chromosomal crystal-protein genes have been reported in some *B. thuringiensis* strains (Carlson and Kolstø, J. Bacteriol. 175: 1053-1060